

INTRODUCTION AND SUMMARY

C-076 B_{1a} is a macrocyclic lactone disaccharide with significant antiparasitic activity. It has been evaluated in a series of acute, subacute, teratogenicity, and mutagenicity tests which are summarized below and presented in detail in the text that follows.

1. C-076 (B_{1a}): Acute Oral Toxicity in Mice and Rats.

The acute oral toxicity of C-076 (B_{1a}) (L-676,895-00P26) was studied in adult female mice of three different strains and in young adult and infant male and female rats.

The oral LD₅₀ values are tabulated below:

Species	Strain	Sex	Vehicle* Conc. %	Date of Study	LD ₅₀ (95% fiducial limits) mg/kg
Mouse	CF ₁	F	0.2	6/7/77	22.2 (15.7 - 31.5)
Mouse	CF ₁	F	0.2	6/8/77	23.8 (14.3 - 39.6)
Mouse	CF ₁	F	0.8	6/13/77	13.6 (7.32 - 25.3)
Mouse	CF ₁	F	0.2	11/18/77	18.3 (10.1 - 33.2)
Mouse	CD-1	F	0.2	11/18/77	17.4 (12.1 - 25.1)
Mouse	ICR (MSD)	F	0.2	11/18/77	18.7 (12.9 - 27.1)
Rat (YA)	CRCD	M	0.2	7/6/77	10.6 (7.67 - 14.5)
Rat (YA)	CRCD	F	0.2	7/6/77	11.3 (7.48 - 17.1)
Rat (I)	CRCD	M,F	varied	7/14/77	1.52 (1.05 - 2.19)

* = Sesame oil; YA = young animal; I = infant

The LD₅₀ values for C-076 (B_{1a}) in adult female mice in all strains tested were comparable. Increasing the concentration of C-076 (B_{1a}) administered from 0.2 to 0.8 percent decreased the LD₅₀ in adult female mice approximately 50 percent. Young rats of both sexes were more sensitive to the acute administration of C-076 (B_{1a}) than adult female mice at comparable drug concentrations. Infant rats were the most sensitive test system.

to 18 of gestation; however, there were too few surviving pregnant females to permit statistical evaluation.

There was no mortality in pregnant females receiving 0.1, 0.5, or 0.75 mg/kg/day of C-076 (B_2). Deaths, preceded by tremors and/or coma, occurred in 1 mouse in each of the 1.0, 2.0, and 8.0 mg/kg/day dosage groups receiving C-076 (B_2). Tremors were observed in 1 mouse at 0.1 mg/kg/day. There were no physical signs of toxicity observed among mice given 0.5 or 0.75 mg/kg/day of C-076 (B_2). Retardation in maternal weight gain from Days 6 to 18 of gestation compared to controls occurred at dosages of C-076 (B_2) equal to or greater than 1.0 mg/kg/day although the decrease was significant ($P \leq 0.05$) only at 8.0 mg/kg/day.

Because malabsorption could explain the lack of a clear dose response in the mortality and toxicity observed, the vehicle for the subsequent teratology studies was changed to sesame oil in which both compounds are soluble.

In two replicate teratology studies 8 groups of 25 pregnant mice each received either C-076 (B_{1a}) or C-076 (B_2) by gavage as a solution in sesame oil daily from Days 6 to 15 of gestation. The dosage levels of both components were 0.1, 0.2, 0.4, or 0.8 mg/kg/day, and two additional groups served as vehicle controls. A dose-related increase in mortality from 0.1 to 0.8 mg/kg/day was observed with both C-076 (B_{1a}) and C-076 (B_2). The incidence of mortality in the 0.1, 0.2, 0.4, and 0.8 mg/kg/day dosage groups was 1, 3, 6, and 8 for mice receiving C-076 (B_{1a}), and 2, 3, 5, and 5 for mice receiving C-076 (B_2). Tremors followed by coma were apparent in all dosage groups with both compounds. There was no

In mice physical signs including ataxia, bradypnea, tremors, decreased activity, and loss of righting were observed within two hours of drug administration and these effects persisted for up to five days. Deaths occurred from 21 minutes to five days postdosing.

In rats physical signs of toxicity were not observed until the day following dosing and included tremors, decreased activity, ataxia, loss of righting, chromodacryorrhea, and chromorhinorrhea at doses of 8 mg/kg and greater. Tremors, ataxia, and loss of righting persisted through the third and fourth days following dosing in these same groups. Deaths occurred between overnight and the fourth day following dosing.

Physical signs of toxicity among infant rats administered C-076 (B_{1a}) were confined to tremors observed at the highest dose administered (32 mg/kg) within one hour of dosing. The majority of deaths in infant rats occurred within 24 hours of drug administration.

2. L-676,895: Bacterial Mutagen Test (Ames Test): TT #76-8052.

C-076 (B_{1a}), identified as L-676,895, was tested for its ability to revert to histidine independence mutant strains of Salmonella typhimurium (TA1537, TA92, TA98, and TA100) that require histidine in order to grow. The tests were done with and without a metabolic activation system prepared from rat liver. The compound was tested at concentrations up to 2000 mcg/plate.

C-076 (B_{1a}) did not produce significant increases in reversion to histidine prototrophy under any of the test conditions at any of the concentrations tested.

The positive control, 1-methyl-2(3a,4,5,6,7,7a-hexahydro-1,2-benzisoxazol-3-yl)-5 nitroimidazole, identified as MK-436, produced the anticipated increases in revertants particularly after metabolic activation except in studies with tester strain TA92 where its effect was variable.

3. C-076 (B_{1a} and B₂): Oral Teratogenic Evaluation in Mice.

TT #76-723-0/-1/-2/-3.

In two range-finding studies, C-076 (B_{1a}) and C-076 (B₂) were administered as aqueous suspensions by gavage to 16 groups of 5 pregnant mice each from Days 6 to 15 of gestation. The dosage levels were 0.1, 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 mg/kg/day of C-076 (B_{1a}) and 0.1, 0.5, 0.75, 1.0, 2.0, 4.0, 6.0, and 8.0 mg/kg/day of C-076 (B₂). Two additional groups served as controls and received the vehicle, 0.5 percent aqueous methylcellulose.

There were deaths, often preceded by tremors and/or coma, in the groups receiving 0.1, 0.25, 1.0, and 8.0 mg/kg/day of C-076 (B_{1a}), and tremors were observed at 4.0 and 6.0 mg/kg/day. No signs of toxicity were observed in the group receiving 0.5 mg/kg/day of C-076 (B_{1a}). There was a nonsignificant ($P > 0.05$) decrease in average body weight gain compared to the control mice from Day 6 to 18 of gestation in the group receiving 1.0 mg/kg/day of C-076 (B_{1a}). There were significant decreases ($P \leq 0.05$) in average body weight gain during the same time period in the 2.0, 4.0, and 6.0 mg/kg/day dosage groups. The mice at 8.0 mg/kg/day also had a decrease in average body weight gain from Days 6

effect on the average maternal weight gain, reproductive status, or average live fetal weight per litter among surviving mice at any dosage level with either compound.

External examination revealed a teratogenic effect as judged by the incidence of cleft palates. Five and 10 fetuses in the 0.4 and 0.8 mg/kg/day C-076 (B_{1a}) groups, respectively, and 2, 5, and 11 fetuses in the 0.2, 0.4, and 0.8 mg/kg/day C-076 (B_2) groups, respectively, had cleft palates. Visceral and skeletal examination produced no further evidence of teratogenicity.

4. C-076 (B_{1a}): Oral Teratogenic Evaluation in Mice. TT #77-705-0.

A highly purified sample of the B_{1a} component of C-076 was used in a mouse teratology study. Four groups of 20 pregnant mice each were administered C-076 (B_{1a}) as a solution in sesame oil once daily by gavage from Days 6 to 15 of gestation at doses of 0.1, 0.2, 0.4, and 0.8 mg/kg/day. Two additional groups of 20 mice each served as vehicle controls.

There were deaths, preceded in the majority of cases by tremors, coma, or both in the 0.1, 0.4, and 0.8 mg/kg/day dosage level groups. There were no adverse effects on average maternal weight gains noted among surviving female mice in any dosage level group. There were no treatment-related effects on the reproductive status of surviving females or on the average live fetal weight per litter in any treatment group.

External examination of fetuses revealed a teratogenic effect in the 0.4 and 0.8 mg/kg/day dosage level groups as evidenced by an increased incidence of cleft palates with 4 and 5 fetuses affected, respectively. Visceral and skeletal examination produced no further evidence of teratogenicity.

5. C-076 (B_{1a}): Ten-Day Oral Toxicity Study in Pregnant Mice.

TT #77-717-1.

In an attempt to establish the threshold of toxicity in pregnant mice, four groups of 20 pregnant mice each were administered C-076 (B_{1a}) as a solution in sesame oil from Days 6 to 15 of gestation. The dosage levels were 0.025, 0.050, 0.075, and 0.10 mg/kg/day, and one group of 20 mice served as a vehicle control. One mouse died at 0.10 mg/kg/day, and tremors were observed in several additional mice at this dose. One 0.075 mg/kg/day mouse had muscular tremors, and this mouse became comatose and was sacrificed while aborting. No mortality or physical signs of toxicity were observed in any other treatment group. Average maternal weight gains of surviving mice were unaffected by treatment at any dosage level.

6. C-076 (B_{1a}): Oral Reproduction Study in Rats. TT #77-706-0.

C-076 (B_{1a}) was administered orally to three groups of 12 female rats each at dosage levels of 0.5, 1.0, and 2.0 mg/kg/day from 14 days before mating, throughout gestation and lactation until Day 21 post-partum. Two additional groups of 12 females each served as controls and received the vehicle, sesame oil, in the same dosing regimen as the treated animals.

The high dosage was reduced after five doses to 1.5 mg/kg/day because of whole body muscular tremors observed in females at 2.0 mg/kg/day. Two of the high dose females died and a third was sacrificed because of its moribund condition after 9 to 15 doses. No physical signs of toxicity

study. C-076 (B_{1a}) had no effect on the mating, reproductive status, length of gestation, or postimplantation survival rates of females at any treatment level.

Pup survival was unaffected by treatment with 0.1, 0.2, or 0.4 mg/kg/day of C-076 (B_{1a}). There were no signs of toxicity among pups from females dosed with 0.1 mg/kg/day of C-076 (B_{1a}). Very slight, infrequent, spastic movements were observed among pups from 7 of 13 litters of 0.2 mg/kg/day. At 0.4 mg/kg/day the frequency and severity of these spastic movements increased with all litters affected. In addition, tremors developed in pups from most of the litters at 0.4 mg/kg/day. Occasional hypothermia and a reduction in the quantity of milk observable in the stomach were also seen in some pups at 0.4 mg/kg/day.

Average Day 1 postpartum pup weights were unaffected by treatment with C-076 (B_{1a}). Average pup weight on Days 7, 14, and 21 was unaffected at 0.1 mg/kg/day. Although there was no apparent overall effect on average pup weight at 0.2 mg/kg/day, there was a treatment-related decrease in average pup weight observed in one litter at 0.2 mg/kg/day. At 0.4 mg/kg/day average pup weights on Days 7, 14, and 21 were significantly reduced ($P \leq 0.05$) compared to controls. Developmental retardation, as evidenced by significant ($P \leq 0.05$) delays in eye opening, ear opening, and hair growth were noted in pups from the 0.4 mg/kg/day dosage level group compared to controls. A slight, but significant ($P \leq 0.05$), decrease in the time to incisor eruption was noted in pups from the 0.2 and 0.4 mg/kg/day dosage level groups compared to the controls.

were observed in other females in this group after the dosage was reduced to 1.5 mg/kg/day. No physical signs of toxicity were observed in females dosed with 1.0 or 0.5 mg/kg/day.

Average body weight gain of females in the prebreeding period in the 0.5 and 1.0 mg/kg/day groups was comparable to that of controls. There was a significant decrease ($P \leq 0.05$) in average body weight gain of females in the 1.5 mg/kg/day dosage group from Days 1 to 8 of the prebreeding period. This decrease was the result of weight losses in the 3 females which subsequently died or were sacrificed when moribund. No adverse effects on body weight gain were noted among females in any treatment group for the remainder of the study. Average body weight gain of females from Days 1 to 7 and 7 to 15 of gestation were significantly increased ($P \leq 0.05$) compared to control at 1.0 and 1.5 mg/kg/day. In addition, in females at 1.0 mg/kg/day the average body weight gain was significantly increased ($P \leq 0.05$) compared to the controls from Days 1 to 21 of gestation.

C-076 (B_{1a}) had no effect on the mating, reproductive status, length of gestation, or postimplantation survival rates of females at any treatment level. There was a treatment-related decrease in the number of live pups per litter on Day 1 postpartum at 1.5 mg/kg/day. Litter size was not affected at 0.5 and 1.0 mg/kg/day. Average Day 1 pup weights were significantly decreased ($P \leq 0.05$) in the 1.5 mg/kg/day dosage group compared to the controls. Average Day 1 pup weights were comparable to the controls at 0.5 and 1.0 mg/kg/day.

There was a dose-related mortality among pups from females at all dosage levels of C-076 (B_{1a}). Survival rates of pups in the 1.5, 1.0,

and 0.5 mg/kg/day dosage groups were 0, 14, and 76 percent, respectively, compared to 98 percent in the pooled controls. Treatment-related deaths in pups began on Day 4 postpartum at 1.0 and 1.5 mg/kg/day, and on Day 8 postpartum at 0.5 mg/kg/day. Rapid wasting and the absence of observable milk in the stomach occurred at all dosage levels. The absence of milk was not considered to be an effect on lactation, since surviving pups in affected litters had apparently normal quantities of milk in their stomachs. Lethargy and intermittent tremors were consistent findings among pups from Days 14 to 20 at the 1.0 and 0.5 mg/kg/day dosage levels.

Pups at all dosage levels showed significant decreases ($P \leq 0.05$) in average weight per litter throughout the study. Developmental retardation, as evidenced by a delay in eye opening, was noted in surviving pups at 0.5 and 1.0 mg/kg/day.

7. C-076 (B_{1a}): Oral Reproduction Study in Rats. TT #77-712-0

C-076 (B_{1a}) was administered orally to three groups of 15 female rats each at dosage levels of 0.1, 0.2, and 0.4 mg/kg/day from 14 days before mating, throughout mating, gestation and lactation, until Day 21 postpartum. Two additional groups of 15 females each served as controls and received the vehicle, sesame oil, in the same dosing regimen as the drug-treated animals.

No mortality, physical signs of toxicity, or adverse effects on body weight gain were noted in F_0 female rats throughout the course of the experiment at any dosage level. Treatment-related slight and occasionally statistically significant ($P \leq 0.05$) increases in average body weight gain of females were seen in all dosage groups throughout the

External, visceral, and skeletal examinations of dead pups revealed no evidence of teratogenicity.

At the conclusion of the study pups were randomly selected for continuation on a 14-week oral toxicity study (TT #77-043-0).

8. C-076 (B_{1a}): Eighteen-Week Oral Toxicity Study in Dogs.

TT #76-073-0.

In an 18-week oral toxicity study, 4 groups of 3 male and 3 female beagles each were administered C-076 (B_{1a}) as a solution in sesame oil at doses of 0.25, 0.5, 2.0, and 8.0 mg/kg/day. Two similarly constructed groups of animals received either tap water or sesame oil and served as controls. At the start of the study the dogs were 26 to 42 weeks of age and weighed 7.5 to 12.5 kg. All dogs were examined 5 days a week for physical signs of toxicity with less detailed observations recorded on weekends and holidays. Animals were weighed twice a week during the study. Routine hematologic and serum biochemical determinations were performed in Drug Weeks 4, 8, 13, and 17 for dogs in the 0.5, 2.0, and 8.0 mg/kg/day groups and in Drug Weeks 4, 7, 12, and 17 in the 0.25 mg/kg/day group. Additional hematologic and serum biochemical studies were performed on 8.0 mg/kg/day dogs in Drug Week 1, and an additional bleeding was done on all dogs in Drug Week 13 for serum electrophoretic analysis. Urinalyses were conducted in Drug Weeks 4, 8, 13, and 18 on dogs in the 0.5, 2.0, and 8.0 mg/kg/day group and in Drug Weeks 4, 7, 12, and 17 for dogs at 0.25 mg/kg/day. There were ophthalmologic examinations of dogs in the 0.5, 2.0, and 8.0 mg/kg/day groups

in Drug Weeks 3, 8, 12, and 16, and of 0.25 mg/kg/day dogs in Drug Weeks 3, 8, 11, and 15. Electrocardiograms were recorded in Drug Weeks 2, 3, 8, 12, and 17 for dogs in the 0.25, 0.5, 2.0, and 8.0 mg/kg/day groups. Additional electrocardiograms were recorded on 8.0 mg/kg/day dogs in Drug Week 1. All surviving dogs were killed and necropsied in Drug Week 18.

There were no deaths or treatment-related physical signs in the 0.25 mg/kg/day dosage group.

One dog died at the 0.5 mg/kg/day level after having received 18 doses. Three dogs at the 2.0 mg/kg/day dosage level died after receiving the third dose, and at 8.0 mg/kg/day, 3 dogs died within 24 hours of receiving the first dose. Signs of toxicity at 0.5, 2.0, and 8.0 mg/kg/day included whole body muscular tremors, ataxia, mydriasis, and ptialism. In addition, at 8.0 and 2.0 mg/kg/day, tonic convulsions and emesis were observed. Dosing was terminated in the 8.0 and 2.0 mg/kg/day dosage groups after the first and third doses, respectively. Mydriasis was observed up to 72 hours after the final dose in dogs in the 2.0 mg/kg/day dosage group and up to five days in dogs receiving a single dose of 8.0 mg/kg/day.

Body weights of dogs in the 0.25 mg/kg/day group were comparable to those of controls throughout the study. Treatment-related decreases in body weight were observed in the 0.5 mg/kg/day dosage group in 2 dogs in Drug Week 3. One of these dogs, mentioned above, was found dead in Drug Week 3. Dosing was suspended in the second dog for a period of four days and thereafter its body weight gain was comparable to that of the control

groups. Dogs at 2.0 mg/kg/day showed decreases in body weight after two doses, but surviving dogs recovered after suspension of dosing. No treatment related changes in body weight were observed in surviving dogs at 8.0 mg/kg/day.

The only treatment-related ophthalmologic change observed was a traumatic corneal scar that occurred in 1 dog in the 8.0 mg/kg/day dosage group as the result of abrasions suffered during tonic convulsions.

There were no treatment-related changes in hematologic or serum biochemical parameters in the 0.25, 0.5, and 2.0 mg/kg/day dosage groups. The 8.0 mg/kg/day dogs showed stress-related changes in hematologic and serum biochemical parameters in Drug Week 1 including slight increases in hemoglobin, hematocrit, erythrocyte count, numbers of nonsegmented neutrophils, and serum glucose levels. There were no other treatment-related serum biochemical or hematologic changes in this dosage group for the remainder of the study.

There were no electrocardiographic alterations noted in the 0.25, 0.5, and 2.0 mg/kg/day dosage groups. At 8.0 mg/kg/day bradycardia and elongation of the QT interval were noted in Drug Week 1. Electrocardiographic recordings of surviving dogs in the 8.0 mg/kg/day dosage group and those of dogs in all other treatment groups were comparable to the control groups throughout the remainder of the study.

Treatment-related gross and histomorphologic changes were seen only in animals which died or were sacrificed prior to the scheduled termination of the study. Fairly diffuse hepatocellular vacuolation (not due to neutral lipid accumulation) was seen in 3 dogs at 8.0 mg/kg/day, 2 dogs

at 2.0 mg/kg/day, and 1 dog at 0.5 mg/kg/day. Edema of the gallbladder was also found in 2 dogs at 8.0 mg/kg/day and 2 dogs at 2.0 mg/kg/day. There were no histomorphologic lesions or organ weight alterations attributable to treatment noted in animals which survived to scheduled termination.

9. C-076 (B_{1a}): Fourteen-Week Oral Toxicity Study in Rats

Following In Utero Exposure. TT #77-043-0.

A three-month oral toxicity study with C-076 (B_{1a}) at dosage levels of 0.1, 0.2, and 0.4 mg/kg/day was conducted in weanling rats that had been exposed in utero to these same dosage levels. At the start of the study the rats were between three and four weeks of age and weighed 23 to 91 gm. The drug was administered to three groups of 15 male and 15 female rats each as a solution in sesame oil and two identically constructed groups served as vehicle controls.

Antemortem studies included detailed physical examinations five days a week with less detailed observations on weekends and holidays, recording body weight twice a week, ophthalmologic examination in Drug Weeks 5, 9, and 13, and hematologic and serum biochemical analyses in Drug Weeks 4, 8, and 12. Postmortem studies included routine necropsy examination of all rats; recording weights of spleen, heart, kidneys, testes, liver, and brain; and detailed microscopic examination of various tissues of selected Control I and 0.4 mg/kg/day rats.

There were no treatment-related deaths or physical signs of toxicity in any dosage group. Prior to the initiation of the study

the average body weights of male and female rats in all dosage groups were significantly less ($P \leq 0.05$) than that of the controls. There were no adverse effects on body weight gain in treated rats, however, the male rats in the 0.4 mg/kg/day dosage group did gain significantly more weight ($P \leq 0.05$) throughout the study than their corresponding controls. There were no ocular abnormalities or hematologic and serum biochemical changes that were related to treatment. No treatment-related gross, microscopic, or organ weight changes were observed.

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